Reference: *Biol. Bull.*, **136**: 355–373. (June, 1969)

STUDIES ON THE BEHAVIOR OF *NASSARIUS OBSOLETUS* (SAY) (MOLLUSCA, GASTROPODA)

MARY CRISP

Marine Biological Laboratory, Woods Hole, Massachusetts 02543,
and
Department of Zoology, University of Cambridge, Cambridge, England, U.K.

Nassarius obsoletus (Say), the American mud snail, is an active and abundant prosobranch on the east coast of the United States of America. It is commonest on muddy shores where streams render the water brackish.

C. E. Jenner, in a series of short abstracts (1956, 1957, 1958 and 1959) has described the way in which up to several thousand of these animals may move in contact with each other over the mud flats at Barnstable, Cape Cod. Such schooling was also mentioned in Dimon's monograph (1905), although she seems to have seen it only rarely at Cold Spring Harbor. Schooling is most apparent when the snails are submerged. With exposure to the air at low tide many of the members of a school bury whilst others continue schooling. Physical contact plays an important role in promoting uniform orientation within a school, and Jenner (1957, 1958) suggests that vision and chemical factors may also be involved. He asserts the importance of water currents in determining the direction of movement of a school, which is in general with or against the stream, although schools were observed sometimes to move across the current and where there was no current.

Jenner (1956, 1957, 1958, 1959) also described a change in the distribution of the populations of *Nassarius* from wide dispersal over the mud banks in the harbor to aggregation into dense clumps two to three individuals deep. The change was observed in four consecutive years, from 1956 to 1959, and took place in late July or early August, towards the end of the period of reproductive activity. An autumn migration of populations of *Nassarius* from the littoral to the sub-littoral has been described by C. H. Batcheldor (1915) and by C. J. Sinderman (1960). During the migration snails move in schools, and accumulate in dense aggregations, often covered by sediment below the level of low tide. There they overwinter in a quiescent state, returning to the mud flats in spring.

The present study of *Nassarius* was undertaken in the hope that laboratory reactions to controlled stimuli could be correlated with the behavior of individuals and schools in the field. Dimon's monograph on *Nassarius obsoletus* included experiments on the reactions of small numbers of individuals to light, gravity and water currents. Her conclusions are not always in agreement with mine. Copeland in 1918 described the positive rheotaxis of *Nassarius* when stimulated by *Fundulus* juices. His study was confined to olfactory stimuli.

Observations

Movements of Nassarius obsoletus *in Barnstable Harbor*

The snails were observed on five occasions on the extensive mud-sand flats of Barnstable which are exposed only at low tide. *Spartina alterniflora* grows on the higher banks and is also covered at high tide.

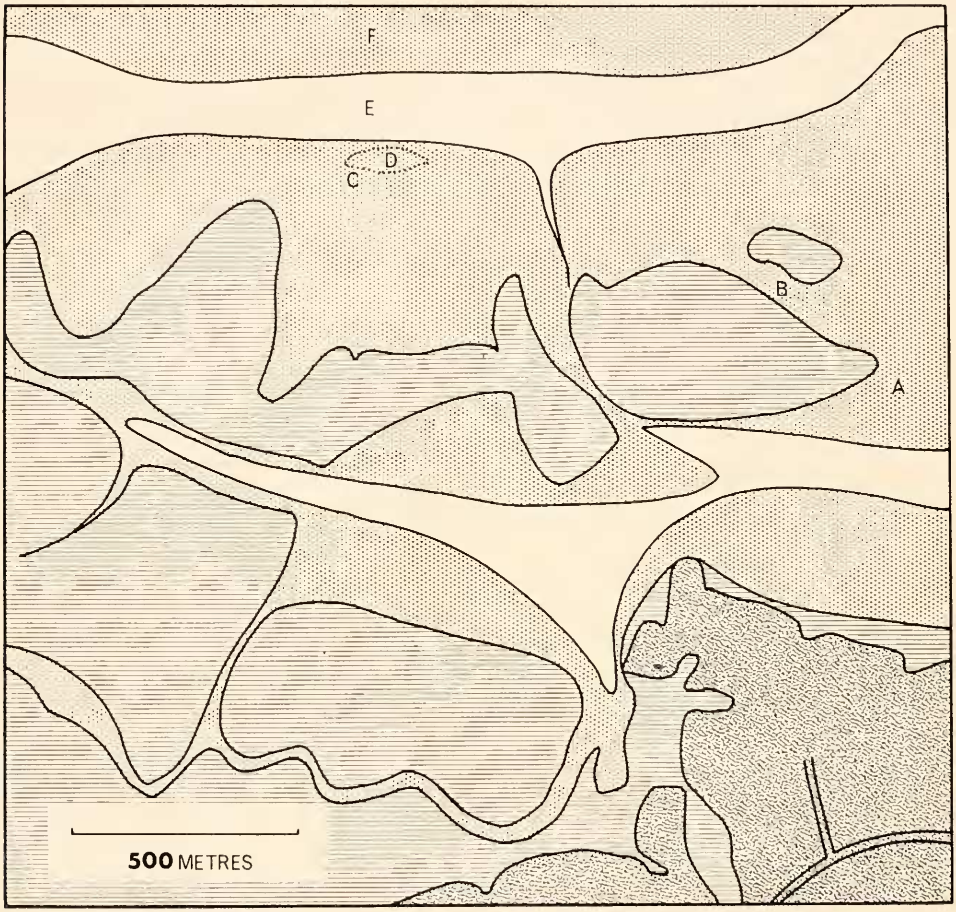

Figure 1. Sketch Map of *Nassarius* Area

A. Source of diatomaceous mud.
B. Source of purple bacteria mud.
C. Shallow stream.
D. Small bank.
E. Main stream.
F. Main bank.
Heavy stipple indicates land.
Horizontal hatching indicates marsh.
Dotted stipple indicates sandy mud banks exposed at low tide.

On 14th July, 1967, during a falling spring tide there were large numbers of *Nassarius* of 1½–2 cm. shell height on the small sand bank shown in the sketch map (Fig. 1). Schools of variable size, often consisting of several thousand individuals moving together and forming a continuous monolayer of snails, were to be seen on the damp surface of the sand bank and just below water level. There were also many individuals wandering freely, not attached to schools. Schools moved in all compass directions, usually downhill in the direction of drainage. Congregations of schools were to be found in pools on the sandbank and at the margins of the bank. When an artificial pool was dug and began to fill with water, many mud snails entered it from the surrounding area in the same way that they entered natural pools. Drainage of a natural pool by digging a channel in the mud resulted in increased activity in the pool with a general tendency of the animals to move downhill. Some animals found the channel and moved down it.

To test the importance of draining water for movement of *Nassarius,* some 40–50 individuals were placed on a dry sloping part of the bank and water was poured over them. They moved downhill into the main stream. Another group placed in a similar position, but not drained with water, stayed still. When the bank became dry, schooling stopped, leaving most of the animals in damp hollows where they partly or completely buried.

As schools reached the edge of the main channel and became submerged the tendency was to move upstream, although exceptions were observed. A typical count taken under 15 cm of water where there was a strong current showed 106 individuals moving upstream to 4 moving down. In the shallow stream a sample had 60 moving up to 1 moving down. Single animals from deeper in the main channel moved uphill at right angles to the current.

On 25th July, 1967, the same bank was observed on an incoming tide. Specimens of *Nassarius* were to be seen in the hollows on the bank and schooling in the surrounding channels. The underwater schools tended to move uphill towards the waterline, and were less closely packed than those on the bank.

On 2nd August, 1967, the bank previously observed was supporting a bloom of purple bacteria and few specimens of *Nassarius* were to be seen. Small numbers were present higher up the shore, moving upstream in runnels draining from the marsh.

On 7th August, 1967, members of the species *Nassarius* were still lacking from the small bank but were abundant higher up the shore, forming a wide band from the shallow stream towards the marsh. The animals seemed generally smaller than the population seen before and included a size group of 4–5 mm shell height not previously noticed. Large numbers of *Nassarius* were present at High Water Neaps level to Mid Tide level among the lowest clumps of *Spartina.* The main banks were more heavily populated than before. On the main banks the snails were concentrated in pools or damper areas whilst firmer clean sandy areas of up to 20 square meters were often free of snails. No orientations were visible as the animals had been exposed for some time so that many had buried.

On 17th August, 1967, mud snails were back in their former abundance on the small bank and were present in vast numbers higher up the shore. In the marsh area small individuals were massed in the upper reaches of drainage runnels in seething aggregations several individuals deep. The aggregations may be similar to those described by Jenner (1956, 1957, 1958, 1959), although the animals he

Table I

Behavior of transplanted Nassarius

Movement of snails with respect to current		Behavior of transplanted snails		
At source of transplanted snails	In area to which transferred	No. moving upstream	No. moving downstream	% UP
A) At low water mark				
Up	Down	27	23	54
Down	Up	21	8	72
Down	Main channel (no native snails)	Random. Tendency to move uphill to waters' edge.		
B) On the marsh				
Up	Down	39	5	89
Down	Up	23	16	59
Down	Down (control)	0	8	0

observed were post-reproductive, whereas the 4–5 mm individuals were the result of the 1967 reproductive activity and not yet themselves of an age to reproduce (see Scheltema 1964). In the marsh areas strong upstream migrations were to be seen, but in the highest reaches some downward migrations occurred. The migrating schools kept close to the interface between mud and water and avoided deeper parts of the channels, walking up the slope to the edge. Where animals moving in opposed directions met, aggregation occurred.

Experiments were performed to see whether the movement of snails upstream in runnels draining off the marsh was a result of conditions in the water or of the physiological state of the animals. Snails were removed from an area in which movement was predominantly upstream and placed in a stream where the local inhabitants had been moving downstream but from which they were removed. The converse experiment was tried for downstreaming snails. The results are shown in Table I.

A proportion of upstreaming animals, especially small ones from the marsh, continued to move upstream in a downstreaming area. Animals taken from an area where the predominant movement was downstream accommodated more readily to moving upstream in an area where others had been doing so. The condition of the animal as well as that of the water seems important in determining the direction taken in a stream. Once the main convoys of snails came into contact with the transferred group the former quickly established dominance, the transplanted animals conforming to the general direction after a brief struggle and blockage.

Mutual conformity

When a *Nassarius* is placed in a dish of seawater and left undisturbed for a few minutes, it goes to the edge of the dish and moves round it in a clockwise or anticlockwise direction. Snails had a greater tendency to turn right at the edge of a dish and move clockwise than to turn left, whether in daylight or total darkness. This tendency was noted also by Dimon, and is probably a consequence of the asymmetry of the animal's gross morphology. Schooling requires the individual to conform with the direction of movement of the group. It is a simple matter to induce a number of snails to follow one another round the edge of a dish, forming a model of schooling behavior.

When another snail was placed in the center, it moved to the edge and was often seen to turn smoothly and follow the direction of movement of those already in procession. Such "invaders" attempting to enter the column in the wrong direction, an event which happened rarely, were soon nudged into conformity or, very rarely indeed, caused all movement in the dish to stop. The results of an experiment in which individuals were introduced singly into a dish containing 7–10 processing snails, are shown in Table II. There is a highly significant tendency for the invader to conform to the direction of movement of those already present, a tendency which may have a bearing on schooling behavior.

TABLE II

Effect of a convoy on snails entering

Direction of movement of convoy	Direction of turning of introduced snail	
	Clockwise	Anticlockwise
Clockwise	25	1
Anticlockwise	1	25
Control (no convoy)	30	22

Not only did an established column of snails influence the turning of newcomers, but the impression was gained that unanimity of direction was more often attained by a group of snails starting simultaneously from the center of a dish than would be expected from the behavior of isolated individuals. Sets of from 2 to 10 individuals were placed in a fingerbowl 17 cm in diameter, filled to a depth of 2–3 cm with seawater. After six minutes the orientation of all the animals lying along the edge of the dish was recorded as clockwise or anticlockwise. Even with the larger numbers in a set it often happened that all were orientated in the same sense. These sets were referrd to as "unanimous," either clockwise or anticlockwise. When a single individual failed to conform with the rest of the set, the result was classified as "all but one conforming," and when more than one individual failed to conform, as "residual." Table III gives the number of sets of animals falling into these categories.

In order to determine whether one individual influences another, it is necessary to compare the results given in Table III with the distribution of orientations to be expected if each individual behaved independently. If p is the probability of turning clockwise and q the probability of turning anticlockwise, then since these are the only possibilities allowed for in the experiment, $p + q = 1$. According to Dimon (1905) this species has a greater tendency to turn right than to turn left; it cannot therefore be assumed that $p = q = \frac{1}{2}$. A large number of trials was made

TABLE III

Effect of number of snails in a dish on conformity

		Number of sets that were:				
I No. of snails per dish	II No. of sets tested	III Unanimous clockwise m = s	IV Unanimous anticlockwise m = 0	V All but one conforming Clockwise m = s − 1	VI Anticlockwise m = 1	VII Residual
Set of 2	48	$\underline{\underline{31}}$ (21.3)	$\underline{\underline{10}}$ (5.3)	7 (21.3)		x
Set of 3	27	$\underline{\underline{11}}$ (8.0)	1 (1.0)	$\underline{10}$ (12.0)	$\underline{5}$ (6.0)	x
Set of 4	27	$\underline{\underline{10}}$ (5.3)	0 (0.3)	$\underline{6}$ (10.7)	$\underline{\underline{5}}$ (2.7)	$\underline{6}$ (8.0)
Set of 5	20	$\underline{\underline{6}}$ (2.6)	$\underline{\underline{1}}$ (0.1)	$\underline{2}$ (6.6)	$\underline{\underline{2}}$ (0.8)	$\underline{9}$ (9.9)
Set of 6	20	$\underline{\underline{8}}$ (1.8)	$\underline{\underline{2}}$ (0.0)	$\underline{4}$ (5.3)	0 (0.3)	$\underline{6}$ (12.6)
Set of 7	19	$\underline{\underline{5}}$ (1.1)	$\underline{\underline{1}}$ (0.0)	$\underline{\underline{4}}$ (3.9)	0 (0.1)	$\underline{9}$ (13.9)
Set of 8	25	$\underline{\underline{4}}$ (0.9)	0 (0.0)	$\underline{3}$ (3.9)	$\underline{\underline{3}}$ (0.1)	$\underline{15}$ (20.1)
Set of 9	13	$\underline{\underline{6}}$ (0.3)	0 (0.0)	$\underline{\underline{4}}$ (1.5)	$\underline{\underline{1}}$ (0.0)	$\underline{2}$ (11.1)
Set of 10	10	$\underline{\underline{2}}$ (0.1)	0 (0.0)	$\underline{\underline{5}}$ (0.5)	0 (0.0)	$\underline{3}$ (9.5)

Values obtained from binomial distribution in parentheses. Values greater than expectation underlined twice. Values less than expectation underlined once.

x indicates impossible situation.

with single individuals to determine how many turned right or left and so to assign values to p and q. Of nearly 300 individuals exactly twice as many turned right as turned left. The same tendency was noted in many of the later experiments and could also be demonstrated in total darkness.

The probability of m out of a set of s individuals in the same dish turning in a clockwise sense will be given by the formula :—

$$Pm = \frac{s!}{m!\,(s-m)!}\,p^{m}\,q^{s-m}$$

if each individual behaves independently.

The above formula was used to obtain values of Pm for $m = s$; $m = 0$ (unanimous situation) and for $m = s - 1$; $m = 1$ (all but one conforming) with $p = 0.667$, $q = 0.333$. By multiplying the calculated values of Pm by the total number of sets (column II), the theoretical number of sets of each category can be calculated. These values are shown in parentheses next to the observed numbers. The table shows that the observed number of sets in which all the snails conformed was much greater than expectation (columns III and IV), while the number in which more than one snail failed to conform (column VII) was less than expectation. A χ^2 test of grouped data indicated a significance level of $P < 0.001$.

Table IV

Effect of "artificial" snails on degree of conformity

Invading snails entering a procession of	No. of invading snails moving		Total
	With procession	Against procession	
Normal living *Nassarius*	80	2	82
Wax-covered live *Nassarius*	38	2	40
Wax-covered empty shells	67	23	90

It might be thought that a snail entering a convoy conformed with its direction of movement merely because it was pushed. Observation of the entering snails did not support this theory, since snails usually glided in unobtrusively, rarely blocking or barging into the column. An attempt was made to analyze the sensory cues enabling a snail to follow the direction of movement of a convoy.

Tactile responses

A series of experiments was performed using empty shells of *Nassarius*, cleaned of algae by boiling in caustic soda and completely covered in paraffin wax, which were suspended in a dish by wires attached to a rotating kymograph drum. The "artificial snails" could thus be moved in convoy at a speed comparable to that of a procession of real snails. The shells were arranged so that they faced forwards when moved anticlockwise. Test snails were placed in the center of the dish and their reactions upon reaching the procession of real or artificial snails at the edge was recorded, noting also, if they conformed to the direction of movement of the column, whether they did so with or without being pushed. No significant difference in behavior was detected between entry into clockwise columns and into anticlockwise columns. Results from the two are therefore not distinguished in Table IV.

Invading snails are seen to conform equally readily to the direction of movement of wax-covered and normal living snails. The coat of wax cannot therefore be responsible for the significantly reduced success of the artificially moved snails in inducing others to conform. The proportion of snails forced into conformity by pushing was higher when artificial snails were used, as shown in Table V.

Artificial snails lack a foot, which may, by touching that of the invading snail, persuade it round; they do not lay a mucous trail; and they might not emit the same scent as real snails.

Table V

Effect of "artificial" snails on manner of achievement of conformity

Processing snails	No. of test snails conforming	
	Without push	With push
Normal live *Nassarius*	27	3
Waxed live *Nassarius*	30	8
Waxed shells	29	38

The possible importance of the mucous trail is demonstrated by a series of experiments in which snails were allowed to process round a dish for 15–20 minutes and were then removed before test snails were introduced singly. The first series was conducted in full knowledge of the sense in which the dishes had been conditioned; in the second series the snails were introduced without foreknowledge of the direction in which the dishes had been conditioned. The two series produced almost identical results, shown in Table VI. It seems that on glass *Nassarius* is able to respond to the direction in which other mud snails have recently been moving. The obvious interpretation is that a mucous trail has been laid down which is polarized in some way that is recognizable to the snail. No visible directionality could be discerned when trails were stained with Methylene blue or Alcian blue and examined under the microscope. Nor could the effect be observed when there was no obstacle corresponding to the edge of the dish against which the snails would turn. A glass ring was temporarily used to confine a procession of snails, and removed. Snails entering the conditioned area did not follow the mucous trail.

TABLE VI

Effect of pre-conditioning a dish

Conditioning of dish	No. of animals turning Clockwise	 Anticlockwise
Clockwise	38 + 44* = 82	8 + 16* = 24
Anticlockwise	16 + 20* = 36	29 + 40* = 69
Controls (dishes not conditioned)	34	17

* Second blind series.

It proved impossible to repeat the experiment on a more natural surface of mud since snails put in to condition the mud ploughed it up and often buried. The significance of conditioning of surfaces by snails in the natural habitat is thus unknown. It is however most important when performing experiments on glass surfaces in the laboratory to obviate any possible interference by mucous trails.

Visual responses

The reaction of *Nassarius obsoleta* to a bright light is variable, and this may account for reports that the animal does not respond to light. However, the animal reacts very clearly to a shadow, although the response soon habituates.

In order to decide whether vision could play a part in schooling or navigation, the animals' response to moving patterns (optomotor response) and to light sources was investigated. The usual type of apparatus involving rotation of a glass dish containing the animal or of a cylindrical pattern around the animal, was employed. The dish was cleared of mucous trails between trials. The results of the experiments are shown in Table VII. In both experiments involving a light compass stimulus the animals tended to maintain station; when the light source was unobstructed (experiment 1) the effect was highly significant, though the animals achieved a constant angle to the light for only short periods of time. The possibility that water currents induced by inertia produced the orientation was eliminated by experiment 3 in which, without a directional light source, no light compass response was given.

The failure of *Nassarius* to follow an optomotor drum with stripes each subtending as large an angle as 60° at the center, renders it most unlikely that behavior leading to schooling involves visual recognition at a distance of other snails and their direction of movement. The light compass reaction may account for the persistence of orientation of schools or individuals when other stimuli seem inconstant or lacking.

TABLE VII

Visual responses

Experiment	Condition	Illumination	Cylinder	Stimulus given	Average rotation to clockwise stimulus	t test	Average rotation to anticlockwise stimulus	t test
1	Moving dish	Side	None	Light compass	+1.08	$P=0.005$	+0.55	$P=0.001$
2	Moving dish	Side	Plain white	Light compass (weak)	+0.21	N.S.	+0.95	N.S.
3	Moving dish	Above	Plain white	None	−0.01	N.S.	−0.04	N.S.
4	Moving dish	Above	60° alternating black and white stripes	Optomotor	−0.04	N.S.	+0.01	N.S.
5	Moving cylinder	Above	60° alternating black and white stripes	Optomotor (no water movement)	−0.20	N.S.	+0.08	N.S.

Values give the number of complete turns (2π) during movement from center to edge of dish. Positive sign implies maintaining station with reference to the rotation of light or pattern.

Rheotactic behavior

Draining slopes On the exposed mud flats, mud snails were observed to move predominantly downhill. This reaction was imitated in the laboratory. A gentle flow of seawater was allowed to run down a plane of glass sheet inclined at a small angle to the horizontal. Batches of specimens of *Nassarius* were placed about two-thirds of the way up the slope, facing in random directions. Direction of movement up or down was noted when individuals had reached the top or bottom of the slope; it was predominantly downhill. After some trials the glass slope was covered with absorbent paper to ensure that movement of the snails down the slope was not due to passive sliding. The result was the same. A wooden slope also elicited the same reaction. A lamp placed near the uphill or downhill end of the slope did not affect results. Predominantly downhill movement was also observed on damp sloping paper with no water flow. Under water the direction of movement on a slope was not consistently up- or downhill being much affected by light, as shown in Table VIII. On the vertical glass sides of the stock tank animals newly put in tended to climb upwards. The same reaction was noted by Dimon who also claimed that *Nassarius* was negatively geotropic in air, contrary to the present findings.

Water currents To test the behavior of *Nassarius* towards a water current in the laboratory, a gentle flow was set up in a fingerbowl of diameter 17 cm filled to a depth of 2–3 cm with seawater, by directing a jet of compressed air along the water surface near one edge of the bowl. The bowl was screened from lateral light by a ring of opaque card and was thoroughly cleaned between trials. Animals were placed singly in the center of the dish, and their reactions recorded in one of two ways. In the first series of experiments, the directions of the initial turn on emerging from the shell, and of the final turn on reaching the edge of the dish were noted. In the second series, using smaller dishes (diameter 10 cm) the total turning of the animal was algebraically summed in units of 45°, clockwise turning being taken as positive, anticlockwise as negative.

TABLE VIII

Effect of slopes

	No. of animals moving	
	Up	Down
1. *In Air*		
With water flow		
Glass slope	12	36
Paper slope	9	143
Wooden slope	2	21
Without water flow		
Damp paper	4	35
2. *In Water*		
Light at upper end	28	4
Light at lower end	7	13

The first series of experiments showed that although the initial turn is not significantly affected by current direction (χ^2 test), the final turn shows a marked tendency of the animals to move with the current. (χ^2 test $P = 0.001$). This was confirmed by the second method which was used in subsequent experiments.

Influence of olfactory stimulants The addition of substances derived from injured animal tissues to water circulating in bowls and to draining slopes causes a reversal of rheotaxis from negative to positive, as described by Copeland (1918). An exactly similar reversal of movement can be obtained when olfactory stimulants are added to water draining down slopes. In most experiments the same individuals were used first as controls in clean seawater and subsequently for experiments with stimulating substances. Positive responses sufficiently clear to establish approximate thresholds were consistently obtained only from snails starved for at least 48 hours in the laboratory. As shown in Table IX, *Carcinus* extract, prepared by crushing a fresh specimen in 10 cc of seawater and filtering the resulting fluid was effective at a concentration of 3×10^{-2} or more in seawater, and so was *Libinia* blood at 1×10^{-4} or more. Water which had been shaken with mud from Barnstable Harbor for some 3–4 hours also reversed the rheotaxis, but water in which 50 mud snails had stood for 3–4 hours in 150 cc had no effect in this experiment. The crab extracts elicited proboscis responses as described by Copeland (1918) and Carr (1967) as well as reversal of rheotaxis. Mud water reversed rheotaxis without eliciting the proboscis reactions at a frequency greater than was shown by controls. Results are given in Table IX.

Influence of physical factors Water containing a concentration of dissolved oxygen greater than that of the water in which the snails had previously been maintained, produced a significant tendency to move upstream, though the reversal of rheotaxis was less consistent than that produced by prey extracts or mud-waters. Batches of snails kept for one hour in seawater saturated with oxygen or nitrogen, and snails kept in aerated seawater were each tested in seawater previously saturated with oxygen, nitrogen or air. Currents were maintained with a nitrogen jet for the nitrogenated and aerated seawater, and with an oxygen jet for the oxygenated seawater. Results are given in Table X. All snails tended to move downstream, but snails transferred to conditions of greater oxygenation showed a significantly higher proportion of individuals moving upstream than did other snails.

TABLE IX

Effect of current bearing olfactory stimuli

Snails in circulating water

Additive	Concentration of extract	No. of snails	% moving upstream
Seawater control	—	20	5
Carcinus extract	5×10^{-5}	14	21
	2×10^{-4}	18	22
	7×10^{-4}	6	50
	1×10^{-2}	19	94
	3×10^{-2}	33	64
Seawater control	—	30	20
Libinia extract	1×10^{-5}	8	38
	1×10^{-4}	8	100
	1×10^{-3}	9	89
Seawater control	—	20	20
Mud water	4×10^{-4}	13	31
	4×10^{-3}	10	40
	1.2×10^{-2}	10	30
	2.3×10^{-2}	10	80
	4×10^{-2}	10	90

Similar results were obtained with snails on draining slopes.

Alteration of salinity to abnormally low or high levels reduces or abolishes the positive rheotaxis normally elicited by *Libinia* extract at concentrations of 1×10^{-4} and above. Normal laboratory tap seawater had a salinity of 31‰. Half strength

TABLE X

Effect of dissolved gases

	% of snails moving upstream in		
	Nitrogen saturated seawater	Aerated seawater	Oxygen saturated seawater
Snails from:			
Nitrogen saturated seawater	33	32	46
Aerated seawater	—	16	—
Oxygenated saturated seawater	11	32	20
Grouping the data		No. moving upstream	No. moving downstream
Snails moved into increased oxygen tension		28	59
Snails moved into same or reduced oxygen tension		14	75

χ^2 for 1 degree of freedom, applying Yates' correction = 5.6.
$0.05 > P > 0.01$.

seawater was prepared by dilution with distilled water. Hypersaline water was prepared by evaporation. Both hyposalinity (15‰) and hypersalinity (33.4‰) reduce or abolish positive rheotaxis, as shown in Table XI.

In 15‰ the behavior of snails is abnormal in that they take longer to extend the foot. During movement the anterior margin of the foot is frequently raised from the substrate and replaced. The whole foot is turned one way and the other to a greater extent than normally, and adheres less firmly to the glass. Scheltema (1965) found that the spontaneous activity of adult *Nassarius* was sharply reduced at salinities of 17‰ and below. In seawater of a salinity of 47‰ the animals emerged but did not stay upright. In seawater of 62‰ the animals remained firmly contracted inside their shells.

These experiments demonstrate the lability of the rheotactic response. Characteristics of the water indicating more favorable conditions, cause an upstream response and vice versa. The presence of food odors represents a more favorable

TABLE XI

Effect of salinity

	% moving upstream	
Salinity ‰	Clean seawater	Seawater with crab extract
15	26	37
28	—	80
31	17	57
33.4	—	20
38.7	—	5
47	Did not crawl	—
62	Remained in shells	—

source and normally causes an upstream movement which would lead to the location of the food. This response can be reversed if unfavorable physical factors are also present. To animals that have recently been fed, however, the presence of food odors does not represent a more favorable condition and they do not usually move upstream.

Since the threshold at which a reversal of rheotaxis occurs varies with the physiological state of the individual, when a population lies at the confluence of a number of streams, those streams with a strong stimulating capacity will attract most of the individuals in their path, while those with a weak odor will attract only individuals with a low threshold. Thus the population will appear to choose the stream with the strongest odor. This phenomenon can be employed to demonstrate the relative stimulating capacity of various effluents in experiments with Y tubes or multiple choice boxes.

Gregariousness

Because of the schooling and aggregation shown in the field it seemed desirable to see whether there was any chemical attraction between healthy mud snails. A four-way choice chamber consisting of four square bait boxes symmetrically disposed about a central square box into which snails were put at the start of an experiment was used. Water flowed into the bait boxes through flasks and out through a stand pipe in the center box. To test whether the attraction was dependent on the effluent produced only by well-fed animals, the water entering two of the bait boxes was lead through flasks containing equal numbers of fed and starved snails, respectively. The other two boxes served as controls being supplied with seawater at the same rate. To test whether the response was dependent on hunger, as was found with prey odors, fed and starved test animals were placed simultaneously in the center box, and were distinguished by painted marks on the shell. Four replicates in which each bait box served in turn for each effluent or control, constituted a balanced experiment, eliminating any inherent differences between the bait boxes. The results of two independent experiments (Table XII) showed that the snails were attracted by *Nassarius* effluent, particularly that from recently fed *Nassarius*. No difference in behavior was shown by fed and starved test animals.

TABLE XII

Gregarious effect

	Fed snails used as test		Starved snails used as test	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Percentage of snails entering bait box containing:				
Fed *Nassarius*	55%	49%	43%	46%
Starved *Nassarius*	14%	30%	23%	13%
Seawater control (mean of 2 boxes)	1%	0%	8%	3%
Left in starting box	29%	21%	18%	35%
Number of snails used	132	118	200	127

TABLE XIII

Effect of mud and feces

	Feces in left bait box	Feces in right bait box
Number entering bait box with mud and feces	2	3
Number entering control bait box	6	3
Number left in starting box	30	38

A further experiment was carried out to test whether the faeces of recently fed *Nassarius*, perhaps still carrying the odor of the food, were responsible for the attraction. This experiment was performed using the standard two choice (Y tube) apparatus. The results are given in Table XIII and show that mud and faeces left by fed snails held no attraction. A check was made of the gregarious response in the two way choice apparatus; 66 entered the box with an effluent from 100 well cleaned snails and only 5 entered the control box. On another occasion the effect was less marked, 119 entering the *Nassarius* bait box and 71 the control box, suggesting that individuals are not always attracted to each other. These experiments are apparently at variance with the result of an earlier experiment described in the section on rheotactic behavior; in which no reversal of rheotaxis was elicited

TABLE XIV

Upstream movement in response to water stood over deposits of different origin

Source of deposit	No. of animals used	% moving upstream
Barnstable Harbor 24. VII. 67		
Nassarius bank		
Surface	10	80*
Sub-surface	10	80*
Submerged	9	77*
Diatom bank	30	66*
Purple bacteria bank	19	26
Laboratory seawater control	48	29
Barnstable Harbor 2. VIII. 67		
Nassarius bank	10	100*
Purple bacteria bank	10	90*
Diatom bank	10	70*
Control Seawater	30	13
Sandwich Marshes		
Surface Mud (brown)	10	80*
Sub-surface Mud (black)	18	78*
Laboratory seawater control	61	34

Key: "Nassarius bank": A small bank of grey silty sand on which *Nassarius obsoletus* was found in quantity between 14.7. and 2.8.67, and after 17.8.67.

"Diatom bank": A bank of firm sand just below the Spartina grass with abundant *Gemma* and *Hydrobia* and rich flora of diatoms, green and blue green algae.

"Purple bacteria bank": Edges of a channel adjacent to the Spartina marsh with diatoms and abundant purple bacteria.

* Significant difference from control.

Table XV

Effluent choice experiments to seawater passed through various deposits

Experiment	Number of animals used	Source of deposit	Percentage moving into effluent from deposit
1	270	Nassarius bank	30%
		Diatom bank	25%
		Purple bacteria bank	44%
		Laboratory seawater control	1%
2	157	Purple bacteria bank	74%
		Laboratory seawater control (3 replicates)	9%

by water in which mud snails had been stored. It is possible that in the choice experiments the animal is presented with a fluctuating olfactory stimulus to which it is more sensitive. Alternatively, the substance which the animals emit may be labile or the response may not always be given.

Influence of substances derived from deposits *Nassarius* shows a reversal of rheotaxis when exposed to water that has been in contact with sandy-mud (see section on rheotactic behavior). However, the response was not reliably given unless the snails had previously been starved in the laboratory. Seawater was stored over bottom deposits for 24 hours or shaken up with them for 3–4 hours and tested for its capacity to reverse the rheotaxis of starved laboratory animals in fingerbowls with a circulation maintained by an air jet (method 1 of previous experiments on rheotaxis). The results given in Table XIV show that deposits from the surface, from the sub-surface, from aerobic or from anaerobic conditions, and deposits widely differing in their microflora, all contain substances that stimulate the snails to move upstream. The only exception was a single sample of a deposit containing large quantities of purple bacteria. It is possible that some incidental physical property of this sample caused it to be unattractive so that the mud factor was masked.

Similar results were obtained by passing seawater through deposits, each in a conical flask, and thence into "bait boxes" of a 2 or 4 way choice apparatus. In two such experiments (Table XV) the snails showed a clear preference for water that had passed through each of three different deposits over a laboratory seawater control; one deposit was from the bank where the mud snails were commonly

Table XVI

Inactivation of seawater containing mud factor by charcoal filtration

Treatment of water	Number of snails used in test	% moving upstream
Stood over Barnstable Harbor mud 2 days and filtered	27	89
Stood over mud 2 days and filtered through activated charcoal	10	20
Laboratory seawater control	26	19

TABLE XVII

Response of Nassarius *to effluent from natural and pre-boiled mud*

Effluent from	Percentage in prey box Experiment 1 (55 animals)	Experiment 2 (46 animals)
Natural mud	70%	89%
Boiled mud	4%	7%
Laboratory seawater control (2 replicates)	13%	2%

found, and the other two from areas from which they were absent. One of these possessed a high content of purple bacteria, and the other of diatoms, *Enteromorpha* and blue green algae.

There was no evidence from either experiment that the deposits on which *Nassarius* congregated yielded the most effective effluents. The mud from the Sandwich area, where *Littorina littorea* occurs in quantity and there are no *Nassarius,* was as effective in reversing the rheotaxis as Barnstable deposits where *Nassarius* is common.

The effective agent in seawater passed over a muddy deposit can be inactivated by filtering the water through charcoal to which it is presumably absorbed (Table XVI), or by boiling the mud beforehand (Table XVII).

Substrate choice

Though a rheotactic response was given to seawater that had been in contact with mud, no reaction to mud factors specific to the areas occupied by *Nassarius* could be demonstrated. Mud snails were therefore offered a choice of various

TABLE XVIII

Substrate selection

Deposits tested	% of *Nassarius* present in:— Exp. 1 whole deposits	Exp. 2 coarse fraction	Exp. 3 sieved fine fraction
Nassarius bank			
Surface intertidal	38*		
Sub-surface intertidal	2	8	4
Surface submerged	22*		
Diatom bank			
Surface	31*	76*	38*
Purple bacteria bank			
Surface	7**	10†	58**
Number of snails in choice experiment	165	50	25

** Rich in purple bacteria.
* Rich in diatom or algal material.
† Rich in detritus material.
The remaining deposits were predominantly inorganic.
For details of deposits see Table XIV.

sediments to see whether they would accumulate on some more than on others. Some 25 or more individuals were placed at the center of a tray containing a number of samples of different deposits each filling a Petrie dish; the dishes and experiments were replicated to ensure adequate balance for positional differences. The experiments were performed mostly in the dark but identical results were obtained in some experiments in the light. The results of three groups of experiments on the three main types of deposit, and on fractions obtained by sieving are given in Table XVIII. Microscopical examination revealed that certain of the surface deposits were rich in diatoms and algae and others in purple bacteria. The subsurface deposits, as expected, were mainly inorganic. As can be seen from the table, the snails accumulated mainly on the diatomaceous deposits, where they were observed feeding, and on the fine fraction of the bacteria-rich deposit, but they avoided the subsurface deposits. Moreover as the surface flora of the deposits was removed by the snails' activity they tended to migrate from the diatom rich sand, where the algae were mainly at the surface, to the muds which were rich throughout in bacteria. As *Nassarius obsoletus* is known to be a facultative scavenger and deposit feeder, the conclusion appears to be that in the laboratory the snails congregate on deposits with the greatest abundance of food rather than on a particular deposit type. Wieser (1956) obtained similar results for the cumacean *Cumella vulgaris*.

Discussion

Before schooling can occur, animals must assemble in a certain locality. The laboratory studies suggest three possible olfactory stimuli which, by causing animals to move upstream, might bring them together at the source of the stimulus.

First, the influence of the attractive factor which is continuously eluted when seawater flows over mud would cause snails to move upstream as far as the point at which a threshold concentration exists, or until opposed by some unfavorable influence. Thus in the sub-threshold concentrations of the mud factor, which might exist near the source of runnels draining off the marsh, snails would move downstream, whereas further down the drainage channels where a higher concentration of mud factor built up, they would move upstream. Downstream movement in the upper reaches of the harbor cannot be attributed to lowered salinity, since the hydrographic study of Ayers (1959) showed that in the area where *Nassarius* is found the salinity departs very little from 31% at any state of the tide.

Secondly, attraction to a localized source of olfactant, for example the effluent from damaged animal tissues stranded on the mud banks, would also lead to an accumulation of snails. This kind of accumulation can be seen on intertidal flats, but the character of such aggregations is apparently different from those described by Jenner (1956, 1957, 1958, 1959). Nor are the majority of schools observed on the flats associated in any way with visible localized sources of olfactant.

Finally, the attraction of individuals by groups of living, undamaged mud snails would be most effective in recruiting snails once a group had been established.

A further reaction likely to lead to congregations of snails is the observed reversal of geotaxis on submergence. By moving down from the damp surfaces of the banks and moving up from below the water level, animals would accumulate at the water's edge in the manner seen in the field.

The orientation of schools was seen to be in the direction which would be predicted from laboratory experiments. For example, schools moved downhill and accumulated in damp hollows on the banks. On reaching the water's edge they moved along it, usually against the current.

In the laboratory much variation of individual behavior towards the same stimulus was observed, whereas the members of a school were seen to move together. In the middle of a school conformity could be purely mechanical, opposition to the direction of movement being physically impossible without disrupting all movement of the school. At the edges, the conforming tendency demonstrated in the laboratory may be important. Any stimulus which induces entering snails to conform with the direction of movement of a school must be polarized in that direction; chemical stimuli are therefore unlikely to be involved. A visual response to the images of other snails at a distance is ruled out by the lack of an optomotor response, although a light-compass reaction to the passing shadows of nearby snails might perhaps occur. The tactile stimulus of contact of the soft parts of the animal, including foot and siphon, is probably of greatest importance in inducing conformity.

Scheltema (1961) showed that the veligers of *Nassarius* responded to seawater which had stood over favorable muds by dropping to the bottom and metamorphosing. The active agent was water-soluble, heat-labile, and unaffected by ordinary filtration. The mud extracts to which adults respond also have these properties. The effectiveness of mud extracts in reversing the rheotaxis of adults is destroyed by filtration through activated charcoal, rendering it likely that the active agent is chemical.

The responses to mudwaters of both larvae and adults may have the function of habitat selection. The adult response to mudwaters was less drastically reduced when they had recently fed than was the response to effluents from damaged animal tissues. Moreover when placed in tissue effluents the snails frequently exhibited proboscis reactions whilst moving upstream. When placed in mudwater the proboscis was extruded with no greater frequency than in clean seawater, suggesting that the positive rheotaxis induced by mudwater may not be primarily a food-seeking response.

My thanks are due to Professor D. J. Crisp for suggesting the problem, to Dr. R. S. Scheltema, to Dr. C. E. Jenner for helpful discussion in the course of the work, and to Dr. M. R. Carriker for kindly reading the text.

SUMMARY

The reactions of specimens of *Nassarius obsoletus* exposed individually to controlled laboratory stimuli are as follows:

1. *Nassarius* shows a light-compass reaction, but no optomotor response.
2. On damp slopes *Nassarius* moves downhill. Submerged *Nassarius* is generally geonegative but its response is altered by a nearby light source.
3. In clean seawater *Nassarius* moves downstream.
4. Addition of effluents from damaged animal tissues or from mud causes a reversal of rheotaxis.

5. A small but significant upstreaming response is elicited by water of raised oxygen concentration.

6. The upstreaming response to olfactory stimuli can be abolished when unfavorable stimuli (hypo- or hypersalinity) are simultaneously present.

7. Water which has passed over living, intact *Nassarius* is attractive to other individuals of the species *Nassarius*, the attraction not being due to faeces.

8. When simultaneously presented with substrates of different kinds, mud snails accumulate on those richest in diatoms and bacteria.

9. The behavior of an individual *Nassarius* is greatly affected by the behavior of others around it. Members of a group of *Nassarius* in the laboratory or in the field show a strong tendency to conform in their direction of movement, thus producing schooling. The orientation of schools in the field was that expected from the behavior of the majority of individuals in the laboratory.

LITERATURE CITED

AYERS, J. C., 1959. The hydrography of Barnstable Harbor, Massachusetts. *Limnol. Oceanogr.*, **4**: 448–462.

BATCHELDOR, C. H., 1915. Migration of *Ilyanassa obsoleta, Littorina littorea* and *Littorina rudis*. *Nautilus*, **29**: 43–46.

CARR, W. E. S., 1967. Chemoreception in the mud snail, *Nassarius obsoletus*. I. Properties of stimulatory substances extracted from shrimp. *Biol. Bull.*, **133**: 90–105.

COPELAND, M., 1918. The olfactory reactions and organs of the marine snails *Alectrion obsoleta* (Say) and *Busycon canaliculatum* (Linn.). *J. Exp. Zool.*, **25**: 177–227.

DIMON, A. C., 1905. The mud snail *Nassa obsoleta*. *Cold Spring Harbor Monogr.*, **5**: 1–48.

JENNER, C. E., 1956. A striking behavioral change leading to the formation of extensive aggregations in a population of *Nassarius obsoletus*. *Biol. Bull.*, **111**: 291.

JENNER, C. E., 1957. Schooling behavior in mudsnails in Barnstable Harbor leading to the formation of massive aggregations at the completion of seasonal reproduction. *Biol. Bull.*, **113**: 328.

JENNER, C. E., 1958. An attempted analysis of schooling behavior in the marine snail *Nassarius obsoletus*. *Biol. Bull.*, **115**: 337.

JENNER, C. E., 1959. Aggregation and schooling in the marine snail *Nassarius obsoletus*. *Biol. Bull.*, **117**: 397.

SCHELTEMA, R. S., 1961. Metamorphosis of the veliger larvae of *Nassarius obsoletus* (Gastropoda) in response to bottom sediment. *Biol. Bull.*, **120**: 92–109.

SCHELTEMA, R. S., 1964. Feeding and growth in the mud snail *Nassarius obsoletus*. *Chesapeake Sci.*, **5**(4): 161–166.

SCHELTEMA, R. S., 1965. The relationship of salinity to larval survival and development in *Nassarius obsoletus* (Gastropoda). *Biol. Bull.*, **129**: 340–354.

SINDERMAN, C. J., 1960. Ecological studies of marine dermatitis-producing schistosome larvae in northern New England. *Ecology.*, **41**: 678–684.

WIESER, W., 1956. Factors influencing the choice of substratum in *Cumella vulgaris* Hart (Crustacea, Cumacea). *Limnol. Oceanogr.*, **1**: 274–285.